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Quercetin ameliorates glucose and lipid metabolism and improves antioxidant status in postnatally monosodium glutamate-induced metabolic alterations

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ABSTRACT

We reported the effects of quercetin on metabolic and hormonal profile as well as serum antioxidant activities in a model of MSG (monosodium glutamate)-induced obesity. Rats were divided into 4 groups: MSG group, submitted to neonatal treatment with high doses of MSG, administrated subcutaneously during 10 days, from 2 day-old; control groups, which received the same volume of saline. After completing 30 day-old, these groups were subdivided into 4 groups: control and MSG groups treated and non-treated with quercetin at doses of 75 mg/kg body weight (i.p.) over 42 days. BW gain and food consumption were higher in MSG treated rats and quercetin significantly reduced BW by 25%. While MSG increased triacylglycerol, total cholesterol and fractions, and reduced HDL concentrations, administration of quercetin normalized HDL-cholesterol and reduced others lipids. Insulin, leptin, glucose and creatinine levels were raised in MSG-treated rats and reduced after quercetin treatment. Alanine transaminase, aspartate transaminase, lactate dehydrogenase and alkaline phosphatase activities were lower after MSG-quercetin combination compared to rats given only MSG. MSG-quercetin combination augmented total protein and urea levels as well as glutathione peroxidase and superoxide dismutase activities in contrast to MSG-treated animals. Quercetin normalized serum lipid and glucose profile and minimized the MSG-related toxic effects, which was associated to its antioxidant properties.

1. Introduction

Eating behavior plays a pivotal role in metabolic and diet-related disorders (Naim et al., 1991). Some mechanisms for food cravings may benefit mammals from nutrient deficiency, otherwise overfeeding of palatable food may cause an imbalanced intake of nutrients (Kaur and Kapoor, 2001). Monosodium glutamate (MSG) is one of the most abundant naturally occurring non-essential amino acids and MSG-treatment is able to produce metabolic changes, which can further result in severe bodily disturbances (Diniz et al., 2005).

The hypothalamus is associated with the control of food intake, energy balance and the autonomic nervous system. Among major causes of neuroendocrine obesity is the hypothalamic lesion-induced obesity (Macho et al., 2000), which can be experimentally induced after s.c. injections of MSG (Nakayama et al., 2003) or via MSG oral administration (Xu et al., 2007) to suckling rodent pups. These animal models for studying obesity often present fasting hyperinsulinemia (Maletínská et al., 2006), hyperleptinemia (Hollopeter et al., 1998), adiposity and increase of plasma fatty acids and triacylglycerols (Dawson et al., 1997). In addition, postnatal administration of MSG in rats seems to be linked to insulin resistance, body weight gain, hyperleptinemia and glucose levels (Iwase et al., 1998; Suga et al., 1999), but the exact mechanisms for these alterations are not yet clearly defined.

Although some variables, such as age, via of administration, dose and period of treatment must be taken account, it is clear that MSG is able to cause metabolic alterations. In this context some studies have shown that MSG induces oxidative stress and hepatotoxicity in rats (Onyema et al., 2006) as well as impaired

Abbreviations: MSG, monosodium glutamate; ROS, reactive oxygen species; SOD, superoxide dismutase; GSH-Px, gluthatione peroxidase; BW, body weight; OGTT, oral glucose tolerance test; TG, triacylglycerol; TC, total cholesterol; HDL, high-density lipoprotein cholesterol; VLDL, very-low-density lipoprotein cholesterol; LDL, Low-density lipoprotein cholesterol; GSH, gluthatione tripeptide. NBT, nitro blue tetrazolium; NBT, nitro blue tetrazolium; PMS, phenazine methosulfate; AST, aspartate transaminase; ALT, alanine transaminase; LDH, lactate dehydrogenase; ALKP, alkaline phosphatase.

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glucose-induced insulin secretion by pancreatic islets of obese mice (Andreazzi et al., 2009). Recent findings proposed that MSG produces altered lipid profile with elevation in reactive oxygen species (ROS) formation and reduction of antioxidant activities (Park et al., 2010).

Quercetin (3,3′,4′,5,7-pentahydroxyflavone), a polyphenolic flavonoid compound occurring mainly in glycosidic forms (Wach et al., 2007), is a potent antioxidant found in vegetables and fruits capable of inducing hepatoprotection and also improving dyslipidemia (Amália et al., 2007). Also, it contains some phenolic hydroxyl groups that have strong antioxidant activity, functioning as a ROS scavenger itself (Boots et al., 2007). Furthermore, growing evidences has pointed to quercetin as a promoter of enhanced superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activities (Amália et al., 2007). To date, no reports have focused e relationship between MSG-toxicity effects and the role of quercetin on dyslipidemia, energy balance, glucose intolerance/insulin resistance and liver metabolism.

Therefore, the present study was carried out to investigate whether quercetin is able to improve nutritional parameters, lipid and hormonal profile, liver and glucose metabolism and serum antioxidant activities of postnatally MSG-treated rats.

2. Material and methods

2.1. Animals and experimental design

Sixty-four male Wistar rats were obtained from the Department of Biochemistry, Bioscience Institute/Campus of Botucatu, UNESP – Univ Estadual Paulista. Animals were housed in polypropylene cages with laboratory-grade pine shavings as bedding and maintained under standard conditions (12 h light/12 h dark cycle; 23 ± 1 °C room temperature). Food standard Purina (3074 SIF, Purina Ltd., Campinas, São Paulo, Brazil) and filtered water were provided *ad libitum*. All animals were divided into four groups (n = 16/group). Control group (CT): fed standard diet and receiving saline as vehicle; MSG group (MSG): given only MSG (4 mg/g body weight) from 2 to 12-day old; quercetin-treated group (CT + QC): receiving standard diet and quercetin at doses of 75 mg/kg body weight (i.p.), started after 30 day-old, over 42 days; MSG-Quercetin group (MSG + QC): receiving MSG, from 2 to 12-day old, and quercetin as treatment, started after 30-day old, over 42 days. MSG was subcutaneously administered at the dose of 4 mg/g body weight (BW) on postnatal days 2–12 (Ebling et al., 1998). At 30 days-old, quercetin at dose of 75 mg/kg BW (Sigma, St. Louis, MO, USA) was dissolved in propyleneglycol as a vehicle and injected intraperitoneally once in a week, followed by 7 days of interval over 42 days, totalizing 6 applications. Food (g) and water (mL) consumption were daily measured and body weights (g) were evaluated each week during all experimental period. Experimental protocols were accepted by Ethical Committee of the Bioscience Institute /UNESP, Brazil, in accordance to the principles of the Canadian Council on Animal Care.

2.2. Biochemical and nutritional determinations

At third week quercetin-treatment were determined fasted glycemic index. After 42 days of treatment (72 days after birth), rats were fasted overnight (12–14 h) and then submitted to oral glucose tolerance test (OGTT). Glucose was orally administered by gavage (3 g/kg) at single dose as 20% aqueous solution, and then glycemia were quantified before administration and at 30, 60 and 120 min after glucose administration. Blood glucose was measured by automatic glucose analyzer (Boehringer Mannheim, Eli Lilly Ltd., São Paulo, Brazil). After OGTT, all rats were anesthetized (0.1 ml sodium pentobarbital 3%, i.p.) and euthanized by decapitation. The blood was placed into centrifuge tubes and allowed to clot to obtain the serum. Triacylglycerol (TG), total cholesterol (TC) and high-density lipoprotein cholesterol (HDL) were determined in serum by enzymatic method (test Kit CELM diagnosis, Modern Laboratory Equipment Company, São Paulo, SP, Brazil). The very-low-density lipoprotein cholesterol (VLDL) was calculated by Friedewald equation and total protein was also quantified (Lowry et al., 1951). Low-density lipoprotein cholesterol (LDL) was selectively precipitated from 60 μl of serum by adding 1 ml of phosphotungstic acid (CELM diagnosis, Modern Laboratory Equipment Company, São Paulo, SP, Brazil) followed by centrifugation at 1400×g for 10 min and finally read at 625 nm. Fat acids determination was achieved from acid-deproteinized samples (phosphate buffer – pH 6.7) containing chloroform. The mixture chloroform-trietanolamine was filtered with sodium diethyldithiocarbamate to form a colored product. Aliquots of serum was used for insulin and leptin determinations through enzyme immune assay kit (EIA kit, Cayman Chemical, USA) using an ELISA reader (Biotech Instruments, INC, USA). GSH-Px (E.C.1.11.1.9.) was assayed using 0.15 M phosphate buffer pH 7 containing 5 mM EDTA, 0.0084 M NADPH, 4 μg of GSH-reductase, 1.125 M sodium aside and 0.15 M GSH. GSH-peroxidase unit was defined as μmol of NADPH oxidized per minute per g protein. SOD (E.C.1.15.1.1.) activity was determined using superoxide radical (O_2^-)-mediated-nitro blue tetrazolium (NBT) reduction by an aerobic mixture of NADH and phenazine methosulfate (PMS). The complete reaction system consisted of 50 mM phosphate buffer pH 7.4, 0.1 mM EDTA, 50 μM NBT, 78 μM NADH and 3.3 μM PMS. One unit of SOD was defined as the amount of protein to decrease the reference rate to 50% of maximum inhibition (Ewing and Janero, 1995). Plasma aspartate transaminase (AST – EC 2.6.1.1), alanine transaminase (ALT – EC 2.6.1.2), lactate dehydrogenase (LDH – EC 1.1.1.27), amylase (EC – 3.2.1.1), and alkaline phosphatase (ALKP – EC 3.1.3.1) activities were determined using kits from Sentinel CH (5-20155; Milan-Italy) and read at 340 nm. Finally, stored serum samples were analyzed for total protein, urea and creatinine concentrations based on kits obtained from Sentinel CH (5-20155; Milan-Italy).

Enzyme activities were performed at 25 °C using a micro-plate reader (μQuant-MQX 200 with Kcjunior software, Bio-Tec Instruments, Winooski, Vermont, USA). Spectrophotometric determinations were performed in a Pharmacia Biotech spectrophotometer with temperature-controlled cuvette chamber (UV/visible Ultrospec 5000 with Swift II applications, Cambridge, England, UK). All chemicals and solvents were purchased from Sigma (St. Louis, Missouri, USA).

Based on food intake and the amount of calories (Seiva et al., 2011), the following parameters were calculated:

$$\text{Energy intake} = \text{mean food consumption} \times \text{dietary metabolizable energy}$$

$$\text{Voluntary food intake}\ (\%) = (\text{mean food consumption} \times 100)/\text{mean body weight}$$

$$\text{Feed efficiency} = \text{mean body weight gain}\ (\text{g})/\text{total food consumption}(\text{g}).$$

2.3. Statistical analysis

The results are presented as means ± standard deviations (SD). Statistical comparisons were performed by two-way ANOVA analysis of variance (two factors: MSG administration and quercetin treatment) complemented by Tukey's test. Statistical significance was set at $p < 0.05$. *Sigma Plot version 11.0* was used for graphic design and statistics.

3. Results

3.1. Nutritional parameters

As shown in Table 1, MSG-treated rats had increased BW gain while quercetin treatment reduced efficiently BW by about 23%. Food consumption, energy intake and glucose levels were significantly ($p < 0.05$) higher in MSG-treated rats and became reduced after quercetin administration. Fat acid levels tended to be elevated after MSG-quercetin combination compared to MSG group (Table 1).

3.2. Glucose and lipid metabolism

All experimental groups responded to the OGTT showing increased glucose levels after 30 and 60 min. After 120 min., the glycemic profile of MSG group was not reestablished to basal levels and was higher than CT group. Quercetin administration in MSG-treated rats reduced significantly glucose levels when compared to MSG group (Fig. 1).

The levels of TG, TC, VLDL-cholesterol and LDL-cholesterol were higher ($p < 0.05$) after MSG administration (Fig. 2). Animals receiving quercetin had significantly reduced TG and VLDL-cholesterol compared to those MSG-QC group. Comparing MSG and MSG-QC groups, quercetin normalized HDL-cholesterol levels and depressed TG, TC, VLDL-cholesterol and LDL-cholesterol levels (Fig. 2).

3.3. Hormone assay

Serum insulin and leptin concentrations were significantly higher ($p < 0.05$) in MSG group than CT group. However, quercetin treatment had reduced the insulin and leptin levels after MSG administration by about 20% and 45%, respectively (Fig. 3A and B).